View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

WS20.1 Target minority bacterial population in cystic fibrosis lung microbiota

P. Sharma[1], N. Stremler[1], M. Reynaud-Gaubert[1], J.-C. Dubus[1], J.-M. Rolain[1]. [1]*Aix-Marseille Univ, URMITE CNRS-IRD-INSERM UMR 6236, Méditerranée Infection, Marseille, France*

Staphylococcus aureus is one of the most frequently encountered species in Cystic Fibrosis (CF) lung microbiota. However its frequent presence on agar plates from sputum samples of CF patients likely impaired the culture of other minority bacterial species that may be responsible for lung exacerbations. In order to discover this minority bacterial population, we examined the sputum sample of a CF patient using conventional culture dependent with or without inhibition of *S. aureus* growth with vancomycin and compare our results to culture independent molecular techniques. Using standard cultures as well as selective media at different physico-chemical conditions, we isolate *Staphylococcus aureus* in the majority of our culture conditions and media. Interestingly, after pre-incubation of the sample with Vancomycin to inhibit *Staphylococcus aureus* growth, we isolate new bacterial species (*Granulicatella, Streptococcus, Rothia, Lactobacillus* and *Propionibacterium* species), especially *Actinomyces radicidentis,* a rare bacterial species associated with endodontic infections never described in the context of CF. Using 16S rDNA PCR amplification and sequencing of subsequent clones, we identified *Streptococcus, Granulicatella, Rothia, Abiotropha, Peptostreptococcus, Lachnospiraceae and Actinomyces* along with *S. aureus* confirming the culture dependent findings. In conclusion, the use of various bacterial inhibitors to inhibit the growth of the dominant bacteria may help to discover the minority bacterial population that could be new or emerging species in the context of CF.

WS20.2 A novel gyrB sequence cluster approach for the identification of multiple CF pathogens

D. Kenna[1], K. Fuentes[1], N. Mustafa[1], K. Martin[1], J. Turton[1]. [1]*Health Protection Agency, London, United Kingdom*

The accurate identification of CF pathogens is critical for surveillance purposes, the monitoring of infection prevalence trends, and for optimising treatment options. In many cases identification by molecular means is a necessity. As a reference laboratory we are routinely required to identify a wide range of CF pathogens submitted from hospitals in the UK. Depending on the organism, CF isolates sent to our laboratory are identified using species-specific PCR assays, sequencing, fatty acid analysis or 16S rDNA sequencing. This multi-faceted approach to identification can be laborious. Our aim in this study was to identify a house-keeping gene common to multiple pathogens, and to develop a sequenced based assay which would facilitate rapid identification. A number of studies have recently highlighted the effectiveness of gyrB as a target for the identification of individual CF pathogens. We therefore aligned gyrB sequences from common CF pathogens and designed degenerate PCR primers which simultaneously detect species of *Achromobacter*, *Ralstonia* and *Pandoraea*, in addition to the *Burkholderia cepacia* complex and *Pseudomonas aeruginosa*. Those isolates producing positive amplicons in this assay are then sequenced and clustered with type strains of these species using BioNumerics software. Sequence clustering with these primers has resulted in clearly defined clusters both of each individual genus, and for species within that genus, and has therefore significantly improved the speed with which we can identify many of these organisms.

WS20.3 Use of matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry for identification of non-fermentative Gram-negative bacteria in cystic fibrosis and non-CF bronchiectasis

A. Lake[1], M. Ellington[2], J.E. Foweraker[3]. [1]*Addenbrooke's Hospital, Cambridge, United Kingdom;* [2]*Health Protection Agency, Cambridge, United Kingdom;* [3]*Papworth Hospital NHS Foundation Trust, Papworth Everard, Cambridge, United Kingdom*

Introduction: Precise bacterial identification is central to infection management and epidemiology. Gram negative non-fermenting bacteria (NFB) causing chronic infection in bronchiectasis undergo phenotypic change, hindering biochemical identification methods. Moreover, these methods may misidentify species from genera rare in other conditions.
Method: We tested 68 NFB from 44 adults (24 with CF and 20 with non-CF bronchiectasis). A subset were selected because biochemical reactions (API 20 NE, bioMérieux) gave no identity, or identified an unusual species. Isolates were analysed by MALDI-TOF (Bruker). National reference laboratory (RL) identification was by PCR or MIDI.
Results: MALDI identified 67/68 bacteria to species level. Formic acid extraction added no benefit. MALDI identification was correct to species level in 38/48 RL-confirmed isolates, with 10 isolates from 8 patients discrepant as follows (RL/MALDI): S*tenotrophomonas maltophilia /Pseudomonas geniculata; Achromobacter xylosoxidans/A.denitrificans; P. alcaligenes/P. oleovorans; Bordetella* sp./no identity; *B. sp/B. bronchiseptica; B.* sp/*B. parapertussis; Aeromonas* sp./*A. caviae*; *P. fluorescens* or *putida/P. monteilii*. MALDI correctly identified all 18 RL-confirmed *P. aeruginosa*, where API 20 NE had given no (5) or wrong (13) results, incorrectly selecting genera such as *Moraxella*, *Comamonas*, and *Photobacterium*. MALDI-TOF analysis is more reliable than API 20 NE in adult bronchiectasis, with great potential for fast, accurate identification in clinical practice.

WS20.4 Real-time PCR (RT-PCR) provides a "window of opportunity": optimal screening of patients with cystic fibrosis (CF) through an earlier detection of *Pseudomonas aeruginosa* (PA)

G. Héry-Arnaud[1,2], E. Nowak[3], F. Le Gall[1,2], S. Rosec[3], J. Caillon[4], V. David[5], I. Danner[6], A. Dirou[7], I. Frachon[8], A. Haloun[6], D. Horeau-Langlard[6], J. Le Bihan[7], M.-R. Munck[9], M.-P. Pelletier[7], J.-J. Quiot[8], S. Ramel[7], G. Rault[7,10], K. Revert[7], E. Poulhazan[3], C. Payan[1,2], R. Le Berre[1,8]. [1]*University of Brest/ Faculty of Medicine of Brest, Laboratory of Bacteriology and Virology – EA 3882 – IFR148, Brest, France;* [2]*University Hospital of Brest, Department of Microbiology, Brest, France;* [3]*University Hospital of Brest, INSERM CIC 0502, Brest, France;* [4]*University Hospital of Nantes, Laboratory of Bacteriology, Nantes, France;* [5]*University Hospital of Nantes, Pediatric Ward, CF Center, Nantes, France;* [6]*University Hospital of Nantes, Thorax Institute, CF Center, Nantes, France;* [7]*Roscoff Hospital, CF Center, Roscoff, France;* [8]*University Hospital of Brest, Department of Internal Medicine and Pneumology, Brest, France;* [9]*University Hospital of Brest, Department of Pediatrics, Brest, France;* [10]*University Hospital of Nantes, CF Expertise Center, Nantes, France*

Objective: To show the faster PA detection provided by sputum culture in conjunction with RT-PCR compared to routine sputum culture alone in the screening of CF patients.
Methods: A French prospective multicenter study (2008–2011) was conducted in three specialized CF centers in Brittany. Only patients PA-free for at least one year were included [Never and Free patients according to the definition of Lee *et al*. (Lee *et al*., 2003)]. For each patient, sputum was collected every three months and clinical and bacteriological analyses were performed. A routine bacteriological culture was performed according to the French standard guidelines. PA was investigated directly on sputa by quantitative RT-PCR targeting the *opr*L gene. Each positive or doubtful *opr*L RT-PCR was controlled by the multiplex *gyr*B/*ecf* X RT-PCR.
Results: Ninety seven patients were enrolled and 757 samples analyzed. By culture, 40 patients were PA-positive: 14 Never patients and 26 Free patients. In 18 cases, both RT-PCR and routine culture were positive. In 20 cases, RT-PCR gave an earlier detection of PA than routine culture alone. In 2 cases, RT-PCR failed to detect PA. The median of time saving achieved through the PCR was 8 months ranging from 12 days to 38 months. The RT-PCR was even more powerful for Never patients (time saving of 16 months *versus* 6 months for Free patients).
Conclusion: This study indicates that PCR assay is an optimal PA screening technique, saving an average of 8 months in early detection over conventional culture. Given that the antibiotic therapy onset may depend on PCR results, we suggest further evaluation of the impact of our PA screening results on CF patient management.